J Comp Physiol A (2006) 192: 341–350
DOI 10.1007/s00359-005-0067-4

ORIGINAL PAPER

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Reproduction elevates the corticosterone stress response in common fruit bats

Received: 13 June 2005 / Revised: 8 August 2005 / Accepted: 10 October 2005 / Published online: 5 November 2005

Abstract Changes in reproductive state or the environment may affect the sensitivity of the hypothalamic-pituitary-andrenal (HPA) axis. However, little is known about the dynamics of the resulting corticosteroid stress response, in particular in tropical mammals. In this study, we address the modulation of corticosterone release in response to different reproductive conditions and seasonality in 326 free-living common fruit-eating bats (*Artibeus jamaicensis*) on Barro Colorado Island in Panama during dry and wet seasons. We present strong evidence that stress sensitivity is primarily modulated by reproductive condition. In reproductively active females, corticosterone increases were more rapid and reached higher levels, but also decreased significantly faster than in inactive females. The corticosterone response was weaker in reproducing males than in females and delayed compared to non-reproductive males. Testes volume in reproductively active males was negatively correlated with corticosterone concentrations. Our findings suggest differentiated dynamics in the corticosterone stress response between sexes, potentially reflecting conflicting ecological demands. In females, a strong acute corticosterone response may represent high stress- and risk-sensitivity that facilitates escape and thus helps to protect reproduction. In males, suppression during reproductive activity could reflect lowered stress sensitivity to avoid chronically elevated corticosterone levels in times of frequent aggressive and therefore costly inter-male encounters.

Keywords Glucocorticoids · Reproduction · Behaviour · Phyllostomidae · Chiroptera

Abbreviations BCI: Barro Colorado Island · HPA: Hypothalamic-pituitary-adrenal · PCR: Polymerase chain reaction · GC: Glucocorticoid

Introduction

Fitness-determining strategic decisions of organisms, considering fundamental demographic as well as behavioural and physiological measures, so-called traits, are central to life-history theory, which aims at understanding their cumulative interaction over the lifespan of an individual to achieve and maximize reproductive success (Ricklefs 2000; Stearns 1992, 2000). As resources are limited, theory argues that investment trade-offs must exist between life-history variables (Stearns 1989; Charnov 1997; Doughty and Shine 1997; Ghalambor and Martin 2001), and that the state of an organism is important for the outcome of such trade-offs (McNamara and Houston 1996). In this study, we address the link between reproductive state and stress sensitivity by measuring corticosteroid

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levels of animals in different reproductive and environmental conditions. As there are both costs and benefits to corticosteroid levels, differences in sensitivity of the hypothalamic-pituitary-adrenal axis ("HPA axis") may reflect a critical trade-off between negative consequences of stress and protection of current as well as future potential reproductive investments.

The release of corticosteroids from the adrenal cortex following the perception of a potential threat is collectively termed the endocrine 'stress response' in vertebrates (Wingfield et al. 1992; Silverin 1998; Sapolsky et al. 2000; Wingfield 2003). It rapidly mobilizes energy reserves, facilitating 'flight or fight' responses (Astheimer et al. 1992; Wingfield et al. 1992). Acute elevation leads to adaptive changes in behaviour and physiology (Wingfield 2003; Wingfield and Ramenofsky 1997; Wingfield et al. 1997, 1998); chronic or excessive exposure, however, may result in reproductive failure and disease (Sapolsky 1992; Heideman 2000). The response is modulated by the HPA axis, and steroid hormones such as corticosterone (CORT) and cortisol, the primary mammalian stress hormones, appear in the blood stream (Eckert et al. 1997; Rich and Romero 2001) with a biosynthesis-related delay of about 3 min (Dallman et al. 1987; Reeder et al. 2004a). Although adaptations of the HPA axis' sensitivity to environmental, life history and social contexts have been shown (Astheimer et al. 1995; Kitaysky et al. 1999; Romero et al. 2000; Goymann et al. 2001; Scheuerlein et al. 2001; Wingfield 2003), little is known about the modulation of the stress response in bats, the second largest order of mammals. In fact, both baseline and post-stress values of glucocorticoid (GC) levels have, to date, been measured in only eight species of free-living mammals (Romero 2002), including one temperate zone bat (Reeder et al. 2004a).

We hypothesize that in the tropics, where chances of future reproductive success are presumably high due to benign climate, low seasonality and more or less constant year-round food availability (for concept see Ricklefs and Wikelski 2002), modulation of individual stress sensitivity may be linked to reproductive investment, contributing to a maximization of individual fitness. Reproduction is costly for bats (reviewed by Racey and Entwistle 2000), yet investment into offspring has not previously been linked on the population level to changes in stress sensitivity in free-ranging tropical mammals. Also, potential differences between the sexes in tropical bats, with temporally discrete investment by females during times of pregnancy and, in contrast to temperate zones, more evenly distributed year-round reproductive activity in males, have not been addressed. We predicted that, as they would trade off the costs of a strong endocrine stress response against investment, reproductively active females would be more stress sensitive than males. If changes in stress sensitivity were induced primarily by reproduction rather than by cyclic environmental fluctuations such as fruiting peaks of canopy trees, we expected such changes to be observed only in individuals that were reproductively active, and not in those that were reproductively inactive.

There are three lines of support for our hypothesis. First, despite their small body size, bats lie at the slow end of the 'slow–fast continuum' of life histories (Promislow and Harvey 1990; Barclay and Harder 2003) and are thought to live longer than most similar-sized non-flying mammals (Tuttle and Stevenson 1982; Racey and Entwistle 2000). It has been suggested that under this slow life history at low latitudes future reproductive success may be valued more than current reproduction because of postponed senescence and may be protected by the trait of high risk sensitivity (Ricklefs and Wikelski 2002), a concept that is supported by birds (see Silverin et al. 1997; Ghalambor and Martin 2001). In tropical bats, glucocorticoid levels are among the highest documented for mammals (Kwiecinski and Damassa 2000). However, most results so far stem predominantly from captive individuals (Widmaier and Kunz 1993; Widmaier et al. 1994; Kwiecinski and Damassa 2000; Reeder et al. 2004b) and data on free-ranging populations are largely lacking (but see Reeder et al. 2004a). Extremely high GC levels may terminate pregnancies (Heideman 2000), triggering a life-protecting emergency life-history stage (Wingfield et al. 1998). Second, reproduction in bats is energetically expensive, yet this investment is not evenly distributed over time as female costs are incurred particularly during late pregnancy and lactation (Harvey 1986; Speakman and Thomas 2003; Korine et al. 2004). Reproductively active males experience costs of harem defence, aggressive inter-male encounters and spermatogenesis (Morrison 1979; Morrison and Morrison 1981; Speakman and Thomas 2003). Due to the low seasonality in the tropics and multiple reproductive events per year, these costs may be experienced year-round (Heideman 1995, 2000; Bernard and Cumming 1997). Third, research on captive flying foxes suggests that female bats may modulate their corticosterone stress response according to reproductive condition (Reeder et al. 2004b).

To examine the influence of reproduction and season on stress sensitivity of free-ranging tropical bats, we quantified circulating blood plasma concentrations of corticosterone in free-ranging common fruit-eating bats (*Artibeus jamaicensis*) in Panama during different reproductive life-history stages and seasons. We tested whether differences in titres represent generally altered stress sensitivity during different stages of reproduction or are primarily a response to seasonal changes in the environment such as changes in food abundance. If differences were directly influenced by season, we expected them to be observed across reproductive conditions in the population, whereas if reproductive condition had a direct influence, we predicted that we would see sharp within-season contrasts between different reproductive conditions.

Materials and methods

Animals and study site

Free-ranging *A. jamaicensis* were caught on Barro Colorado Island (BCI), a field station of the Smithsonian Tropical Research Institute (STRI) in the Republic of Panama (9°10′N, 79°51′W), from 3rd March to 4th April (dry season) and from 1st October to 1st December 2003 (late wet season). Seasonality in the semi-deciduous tropical rainforest on BCI is low in terms of temperature, but distinct in terms of rainfall (Foster and Brokaw 1982; Wright and Cornejo 1990; Leigh 1999). BCI has an annual rainfall of 2,623 mm (Windsor 1990) and is located halfway along a rainfall gradient that stretches across the Isthmus of Panama, with ~1,800 mm of annual precipitation on the Pacific and ~3,000 mm on the Caribbean side. There is a pronounced dry season from late December to early May and a wet season from mid-May to mid-December, with an average monthly rainfall of ~50 and ~285 mm, respectively (Windsor 1990; Paton 2001). Annual temperature averages 27°C. This environmental setting has been shown to fine-tune the timing of reproduction in both birds and bats (Kalko et al. 1996a; Kalko 1998; Wikelski et al. 2000; Thies and Kalko 2004). In this article we define seasonality by rainfall and canopy fruiting activity. While the dry season period is characterized by low rainfall and high food availability, the wet season period experiences considerably more rain but low fruiting activity of canopy trees (Foster and Brokaw 1982; Kalko 1998).

Artibeus jamaicensis is a medium-sized (45 g) bat that feeds almost exclusively on canopy fruits (Morrison 1978; Kalko et al. 1996b; Korine et al. 2000). It has been the subject of extensive long-term studies on its demography, natural history and population ecology on BCI, making it one of the best studied bat species in the tropics (Bonaccorso 1979; Handley et al. 1991; Kalko et al. 1996a; Kalko 1998). Its reproductive cycle is related to the fruiting activity of canopy trees (Kalko 1998). Reproduction in females involves delayed embryonic development and is characterized by a postpartum oestrus (Fleming 1971). Female *A. jamaicensis* give birth to a single young one twice a year. Births are synchronized within the population and take place within a distinct time frame of a few weeks. Adults form harems of 3–30 females with a single adult male in hollow trees (Morrison 1979; Handley et al. 1991). Once adulthood is reached, survival of individuals is high (Handley et al. 1991); the oldest animal of known age was recaptured in a 10-year study on BCI by Charles Handley and was at least 9 years old (Kalko, unpublished results).

Capture, sampling and stress exposure

We captured a total of 326 bats using ten mist nets (6 or 12 m long, 2.5 m high, with four shelves) that were set on the ground and at canopy level in the rainforest. Nets were checked continuously from dusk until dawn. Whenever a bat was caught it was transferred to a nearby field camp in a soft cloth bag. Animals were subsequently measured, handled and sampled under a standardized stress protocol. A small blood sample of 90–300 µl (< 0.75% of body mass) was obtained by puncture of the wing vein with a 27G hypodermic needle. The time elapsed from net extraction to completion of bleeding was recorded to account for the time span from the onset of handling stress. We considered the period from extraction from the net to the completion of bleeding as the stress exposure period. Animals experienced random durations of stress exposure, which were then assigned to categories of < 3 min (baseline), 3–15, 16–30, 31–45, 46–60 and > 60 min. Blood was collected into sodium-heparinized microhaematocrit capillary tubes (Fisher). Blood flow was stopped by pressing a cotton bud onto the small wound. Immediately after sampling, blood was transferred from the capillaries into 1.5 ml PCR vials (Eppendorf) that were stored in ice water in a two-compartment, custom-made field container at 1°C for a maximum of 24 h. Complications from bleeding were never observed, which we confirmed by observation for 72 h in a flight cage of several individuals after they had been bled and by marked recaptures that we obtained in the field within 48 h after sampling. In males, testis size was measured to the nearest 0.1 mm with calipers. Body mass was determined using a Pesola spring balance (0.1 g accuracy). Females were considered reproductively active when they were (a) pregnant, i.e. an embryo could be located by external examination or there was endocrine evidence from quantification of sex steroids (progesterone, 17β-estradiol), (b) lactating, which was determined by stimulating milk excretion from the nipples or (c) postlactating, when there was no milk but nipples were still swollen and regrowth of hairs on the bare spot surrounding the nipple had not yet begun. In males, reproductive activity was indicated if descended testes were enlarged. Afterwards, bats were fed a 5% sugar solution ad libitum, marked with a numbered stainless steel ballchain necklace and released at the capture site. Baselines for corticosterone were obtained by pooling data from individuals that were bled within 3 min after capture.

Hormone analysis

Sample preparation

Samples were centrifuged in the lab on BCI at approximately 2,000*g* for 5 min (Mini Centrifuge, National Labnet, NJ, USA). Plasma was measured by microlitre syringe (Hamilton, Bonaduz, Switzerland) and stored at −20°C until it was transported on dry ice to the University of Ulm, Germany, for analysis. Steroids were extracted from 50 to 150 µl of plasma twice with redistilled dichloromethane (DCM), then dried under a nitrogen stream and resuspended in 5% ethyl acetate (EA) in isooctane (IO). Celite columns (diatomaceous earth) were prepared. The lower phase (0.5 ml) consisted of a mix of 6 g celite and 2 ml doubly distilled water, while the upper phase (1.5 ml) was stuffed with 14 g celite and 7 ml 1:1 1,2-propandediol:ethylene glycol. Resuspended steroids were transferred onto the column soaked in IO and separated based on polarity by increasing stepwise the amount of EA in the mix. This was done to exclude potential cross-reactions of the antibody in the subsequent assay with other glucocorticoids. Corticosterone was extracted with 50% EA in IO. The solvent mix was evaporated; the steroids were resuspended in DCM and then extracted repeatedly to prevent any glycols present from interfering with the radioimmunoassay (RIA). The DCM was evaporated and contents resuspended in steroid assay buffer. A small amount of tritiated corticosterone tracer ([1,2,6,7-^{3}H(N)], Perkin Elmer, Belgium, NET-399) was added to the initial sample and a recovery was taken before and after the extraction and separation process. Assuming equal behaviour of marked and unmarked steroids, this allowed for a calculation of losses during the extraction and separation process.

Assay

Corticosterone was quantified in duplicate by direct radioimmunoassay with a highly specific antibody from Endocrine Sciences (Calabasas Hills, CA, USA, B3-163). Unbound steroid was adsorbed by activated dextran-coated charcoal and removed by centrifugation. Corticosterone concentration in the initial sample was determined by comparison with an assay-specific standard curve indicating the amount of bound radioactive steroid.

Controls

In each of the ten assays performed, there were two or four zero standards (water blanks) and standards of pooled bat plasma containing a known amount of corticosterone (Sigma-Aldrich, Munich, Germany, C2505). As a conservative estimate, the first detected value outside the 95% confidence intervals of the zero standard was accepted as the lower detection limit. CORT was high in all samples; therefore the detection limit was also high (5.19 ± 1.65 ng/ml). Theoretical sensitivity was < 0.1 ng/ml. Blanks were always below detection limit. Inter- and intra-assay variations in the ten assays were 14.6 ± 3.3 and 7.7 ± 3.8%, respectively.

Statistical analysis

The data were normally distributed; therefore parametric statistics were applied. Individuals were assigned to time intervals of 15 min from the time of capture to address temporal effects in the dynamics of the stress response. We used univariate GLM (General Linear Model) with Tukey HSD post hoc pairwise comparisons in an unbalanced design to test for effects of sex and time categories in relation to levels of corticosterone (Fig. 1). After initial analyses, the data were restructured into 30 min time categories to allow for a direct comparison of categories reflecting pre-stress, peak and post-stress levels of corticosterone. Within each time category, comparisons of reproductively active and inactive animals were done by one-way ANOVA (Fig. 2). We used a linear regression model or in case of increase rates of corticosterone a non-linear regression to assess the relationship between absolute time values and corticosterone. The relationship between testis volume and levels of corticosterone was addressed by parametric correlation as both variables were measured with error. Deviations in sample sizes from $N = 326$ are explained by missing data leading to an exclusion of cases from a particular analysis. Data analysis was performed using SPSS 11.01 for Windows. The significance level α was set at $P = 0.05$.

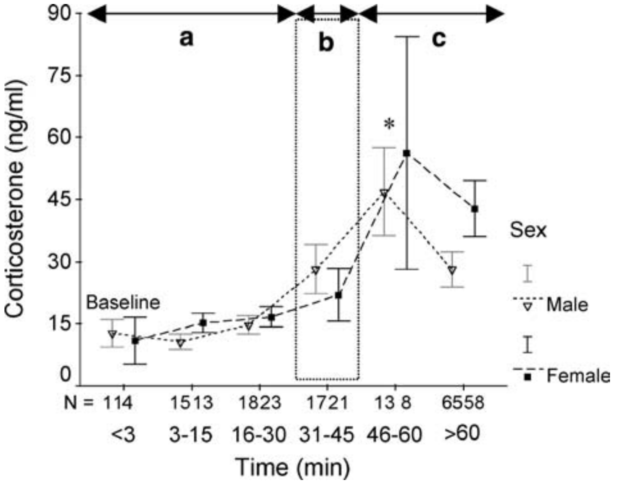

Fig. 1 Circulating plasma corticosterone concentrations in male and female common fruit-eating bats in response to capture and handling in six time intervals after capture. The < 3 min category represents baseline values obtained from animals immediately bled at the net directly after capture. There are three distinct phases of corticosterone response indicated: *a* baseline concentrations, *b* increase phase and *c* maximum levels with subsequent decline. Pregnant females were not included in this analysis. For significances between time categories, see text. Each *bar* represents the mean ± SEM for the sample sizes indicated

Results

Pregnant females were caught only during the first field season (Table 1), which encompassed the end of the dry season and the first birth peak. Lactating females were exclusively caught at the beginning of the second field season within the wet season, after the

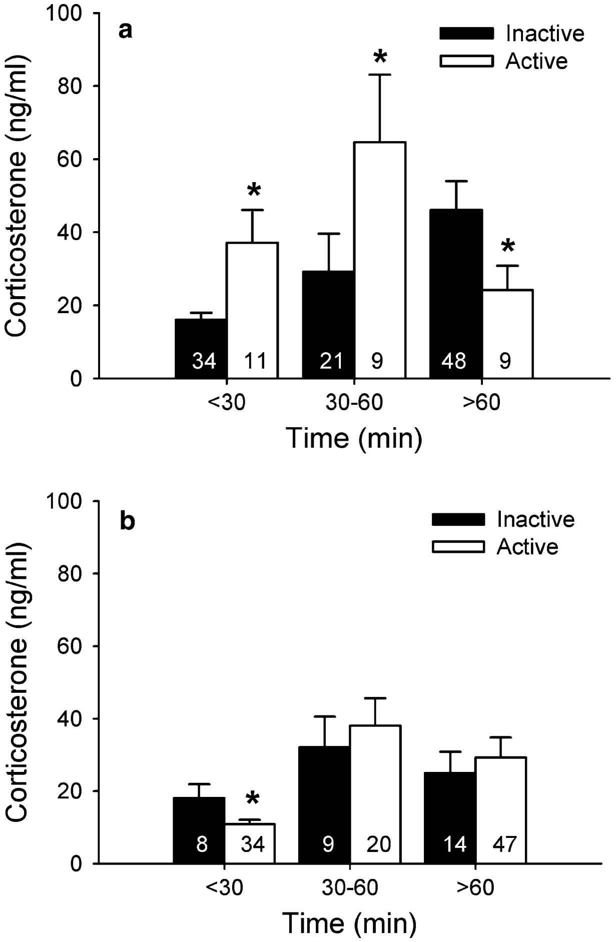

Fig. 2 Corticosterone stress response of **a** female and **b** male *A. jamaicensis* given in time categories and presented separately for reproductive conditions. Reproductive activity included **a** all pregnant, lactating, and postlactating females and **b** all males actively undergoing spermatogenesis as assessed by presence of visibly descended testes. Significances are $P = 0.001$, $P = 0.026$ and $P = 0.041$ for females and $P = 0.024$ for males, respectively. Other within-time category comparisons were non-significant. Each *bar* represents the mean ± SEM for the sample sizes indicated

second birth peak of the year. Reproductively active males, as assessed by testis volume, were encountered in both seasons. However, in the dry season all but one male were reproductively active whereas in the wet season only about two-thirds were reproductively active.

Effects of sex and time

Corticosterone baselines did neither differ significantly between female and male *A. jamaicensis* ($F_{1,14} = 0.48$, $P = 0.50$), nor was there an influence of sex on the time course of corticosterone levels during handling and restraint ($F_{5,275} = 0.35$, $P = 0.86$). However, common fruit-eating bats exhibited a significant time course effect in corticosterone, i.e. a rise in circulating blood plasma concentrations from basal to stress-induced levels over time ($F_{5,275} = 4.52$, $P < 0.001$). Differences in corticosterone levels between time categories were significant for the < 3, 3–15 and 16–30 min categories ($P < 0.014$, $P < 0.012$ and $P < 0.004$, respectively) compared to the 46–60 min category, but there were no significant differences between any of the categories and the 31–45 min category or the > 60 min category (Fig. 1). As there were no significant differences between the < 30 min time categories, we pooled those categories and addressed differences between reproductively active and inactive individuals in 30 min intervals.

Reproductive condition

Corticosterone release was influenced by reproductive condition with pronounced differences between females and males. In reproductively active females, corticosterone release within both the first 30 min ($F_{1,43} = 12.11$, $P = 0.001$) and the 30–60 min ($F_{1,31} = 4.09$, $P = 0.026$) intervals peaked at significantly higher levels than in non-reproductive individuals. In contrast, levels in reproducing females were significantly lower than in non-reproductive females at > 60 min ($F_{1,31} = 4.24$, $P = 0.041$; Fig. 2a). Corticosterone levels of reproductively active males were lower than levels of inactive individuals within the first 30 min time category ($F_{1,43} = 5.46$, $P = 0.024$), but did not differ in either the 30–60 min ($F_{1,29} = 0.21$, $P = 0.65$) or > 60 min categories ($F_{1,62} = 0.17$, $P = 0.68$; Fig. 2b). When addressing the different stages of reproduction in females (pregnant, lactating, postlactating) separately, the strongest stress response was observed in pregnant females. We thus contrasted pregnant females with all other individuals, which revealed that pregnant females stood out in both absolute corticosterone titres (Fig. 3a), and calculated increase rates (Fig. 3b). In males, corticosterone titres were negatively correlated with testis volume, i.e. males

Table 1 Percentage of individual *A. jamaicensis* caught in dry and wet seasons, respectively, by sex and reproductive condition

Sex	Dry season		Wet season	
	♂ ($N = 34$)	♀ ($N = 35$)	♂ ($N = 117$)	♀ ($N = 134$)
Reproductively inactive	2.9	48.6	30.8	90.3
Reproductively active	97.1	–	69.2	–
Pregnant	–	22.9	–	0
Lactating	–	28.5	–	0.7
Postlactating	–	0	–	9.0

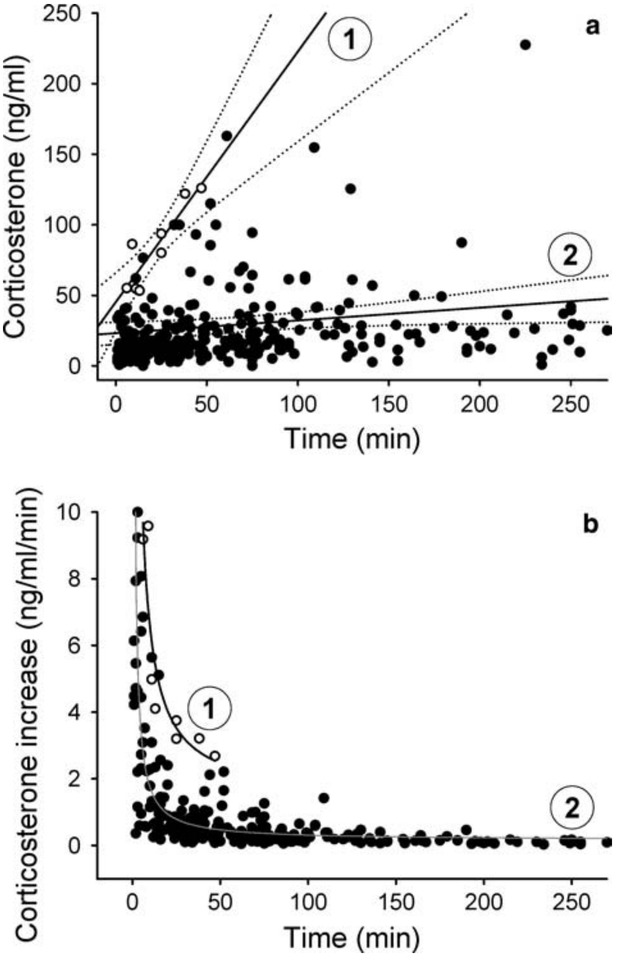

Fig. 3 **a** Correlation between circulating plasma corticosterone concentrations and time from net extraction and **b** baseline-corrected increase rate of corticosterone over time in *1* pregnant female (*open circles*, $N = 8$) and *2* reproductively inactive, lactating, postlactating or male *A. jamaicensis* (*filled circles*, $N = 264$). Correlation coefficients and significances for (a) linear and (b) non-linear regressions are $r_p = 0.82$, $P = 0.002$ (a/1); $r_p = 0.02$, $P = 0.026$ (a/2); $r_p = 0.61$, $P = 0.002$ (b/1); and $r_p = 0.81$, $P < 0.0001$ (b/2)

with larger testes had lower corticosterone levels (Fig. 4).

Seasonality

Season per se did not lead to differences in corticosterone release. In females (Fig. 5a), however, reproductive activity in the dry season was associated with significantly higher postcapture corticosterone levels than in non-reproductive individuals ($F_{1,34} = 8.04$, $P = 0.008$). In the wet season, there was no difference ($F_{1,130} = 0.25$, $P = 0.62$). In males (Fig. 5b), there were no significant differences within the dry ($F_{1,34} = 0.09$, $P = 0.77$) or the wet season ($F_{1,116} = 0.50$, $P = 0.48$), but as virtually all males were reproductively active in the dry season, the dry season comparison relied on the value of only one non-reproductive individual.

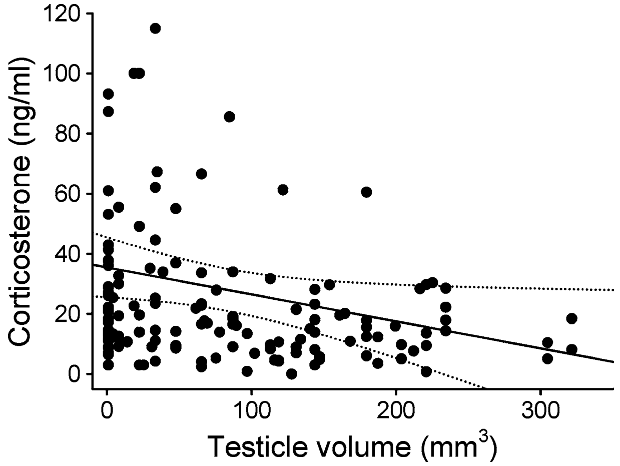

Fig. 4 Correlation between male testicle volume and stress-induced corticosterone levels in reproductively active *A. jamaicensis* ($r_p = -0.17$, $P = 0.046$, $N = 147$). Individuals randomly experienced different durations of stress exposure, thus effects on CORT were randomized

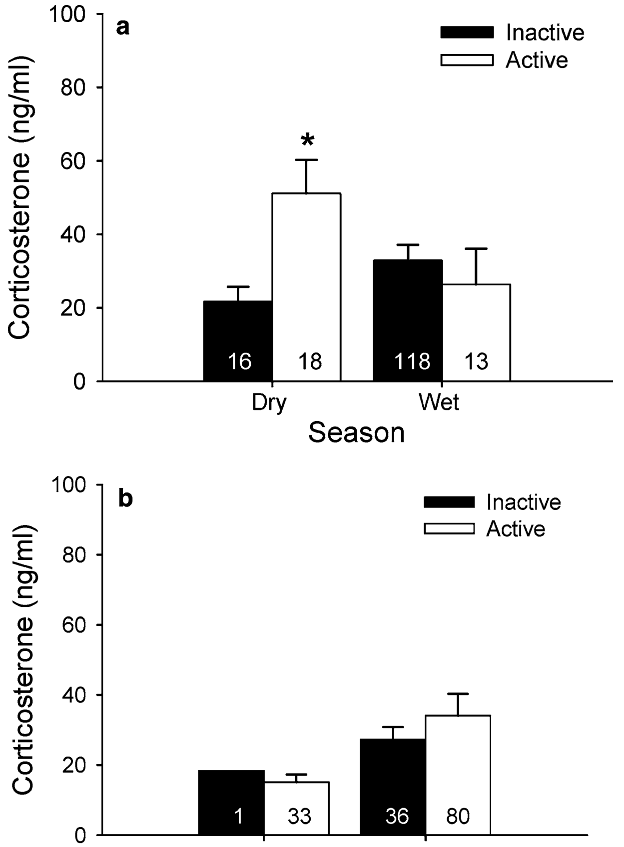

Fig. 5 Corticosterone stress response of **a** female and **b** male *A. jamaicensis* by season and reproductive conditions. Reproductive activity included **a** all pregnant, lactating or postlactating females and **b** all males actively undergoing spermatogenesis as assessed by the presence of visibly descended testes. Significance is $P = 0.008$ for females in dry season. Other within-season comparisons were not significant, as far as sample size allowed for calculation. Each *bar* represents the mean ± SEM for the sample sizes indicated

Discussion

Circulating blood plasma corticosterone concentrations of common fruit-eating bats increased significantly in response to stress exposure and declined after a maximum level had been reached. This response and its dynamics varied significantly with reproductive condition in both females and males. It was stronger, more rapid and less sustained in reproductively active than non-reproductive females, whereas in males, the acute response to stress was delayed in reproductive compared to non-reproductive individuals. Stress sensitivity appeared to be depressed in the short term. As we only found non-significant differences in female corticosterone levels between non-reproductive animals from both seasons and as reproduction had a significant influence on the within-season corticosterone stress response in both males and females, we conclude that reproductive condition, rather than seasonality of the environment, may be a major determinant of the dynamics of the corticosterone stress response. Both sexes appear to be capable of modulating the corticosterone stress response to meet different ecological demands at different life-history stages, namely changes in reproductive condition.

Gender, temporal effects and corticosterone levels

Glucocorticoid levels increase in bats in response to stressors such as handling and restraint. Previous studies emphasized the role of cortisol as a primary mammalian stress hormone in bats (reviewed by Kwiecinski and Damassa 2000). Recent findings in free-living little brown bats (*Myotis lucifugus*, Reeder et al. 2004a), however, also suggest an important and probably even central role for corticosterone in relation to reproduction. In the absence of data on either glucocorticoid in our model system, we were encouraged to choose corticosterone for this study, and our data indeed largely support this choice. *A. jamaicensis* shows a characteristic, sex-independent stress response with corticosterone levels rising from a baseline to a maximum followed by a subsequent decrease. Surprisingly, the corticosterone response in *A. jamaicensis* was less rapid than in *M. lucifugus* (significant 15 min after capture) and captive flying foxes, *Pteropus hypomelanus* (significant after 20 min of restraint; Widmaier and Kunz 1993; Reeder et al. 2004b).

Why is the corticosterone stress response in *A. jamaicensis* delayed compared to the other two species studied in subtropical and temperate settings? First, the stimulus itself differed between studies. In the previous studies by Reeder, Widmaier and colleagues, the animals had been physically restrained, while we kept our bats in cloth bags, shielding them from visual cues and allowing some freedom of movement. A stronger stimulus in these studies may therefore have elicited a more rapid and intense corticosterone release.

Second, bats living in the tropics may in general be more stress sensitive than temperate species, thus the response may be delayed to avoid a strong immediate build-up when higher overall corticosteroid levels are reached (reviewed by Kwiecinski and Damassa 2000). This mechanism may protect fitness in settings with year-round reproductive activity, multiple reproductive cycles and an assumed high predation pressure (Ricklefs and Wikelski 2002). Interestingly, flying foxes in general reach higher peaks in corticosterone levels than microbats and most other mammals (Kwiecinski and Damassa 2000), suggesting high susceptibility to the negative effects of stress and elevated overall sensitivity of the HPA axis. Our results suggest that there might even be a tropical syndrome of high stress sensitivity in bats spanning Old and New World tropics.

Third, corticosterone might be co-secreted with other GCs such as cortisol but might serve a slightly different physiological function, thus being produced with a different time delay, possibly explaining the unusual secretion pattern. Despite the delay, our data still fit into the overall temporal and concentration patterns of the endocrine stress response in vertebrates (see Wingfield et al. 1992; Koolhaas et al. 1997; Berger et al. 2005). Most importantly, the biological relevance and the biological effects of the temporal dynamics discussed here on fitness need to be examined in future studies to fully understand their functional importance.

Reproductive condition

Reproduction has a profound influence on corticosterone levels in *A. jamaicensis*. Although data available on corticosteroid levels of free-ranging mammals in different reproductive conditions are scarce, elevated plasma corticosteroid levels have been found in a range of mammals including little brown bats (*M. lucifugus*, Reeder et al. 2004a), spotted hyenas (*Crocuta crocuta*, Goymann et al. 2001), yellow-pine chipmunks (*Tamias amoenus*, Kenagy and Place 2000), degus (*Octodon degus*, Kenagy et al. 1999) and golden-mantled ground squirrels (*Spermophilus saturatus*, Boswell et al. 1994).

Why do corticosterone dynamics differ between sexes and reproductive conditions in *A. jamaicensis*? First, reproduction is energetically expensive, so elevated corticosteroid levels may facilitate energy availability via an up-regulated metabolism. However, if high levels were constantly required and a higher baseline had to be maintained, we would expect both to constantly range above those of non-reproductive individuals. Second, the observed increase in corticosterone may be an expression of an avoidance strategy, increasing survival and protecting future reproduction. Females that are more easily stressed, and already energetically challenged, would be highly sensitive to hazards and avoid even the slightest trace of risk. Third, circulating GCs can also be bound to a considerable extent to gonadal steroid-binding globulins, which are increased by reproductive activity. The total GC concentration may be higher, resulting from an increased capacity of steroid-binding globulins expressing certain affinities to GCs (for review see Breuner and Orchinik 2002). This might also be an explanation for why GC concentrations vary significantly across the females' oestrous cycle, as changing concentrations of estrogens and progesterone are known to influence the expression and occupancy of plasma steroid-binding globulins.

Glucocorticoid levels may thus potentially serve conflicting interests, increasing survival on the one hand and reproduction on the other, including current as well as future potential reproduction (Greenberg and Wingfield 1987; Wingfield 2003). High glucocorticoid concentrations may be beneficial because they support metabolism and biosynthesis, and thus in the short term facilitate escapes that increase the likelihood of survival of the bats as well as their offspring. Higher GC levels may also lower the risk of autoimmune diseases (review by Raberg et al. 1998). However, high levels of GC may also be costly (Sapolsky 1992; Sapolsky et al. 2000; Wingfield et al. 2000), as they could increase the risk of mortality and reduce chances of future reproduction, especially if they are maintained for longer periods of time. Glucocorticoids not only have the potential to trigger emergency responses directed towards the survival of individuals but can also lead to loss and abandonment of offspring (Wingfield et al. 1998). Reproduction in female common fruit-eating bats is a life-history stage in which high sensitivity to stress may thus have benefits. However, if a strong stressor is actually experienced, leading to a rapid increase in plasma levels of corticosterone, counterbalancing controls may be critical to prevent stress-induced loss of the embryo. There is evidence of termination of pregnancy following strong stress exposure in various species of bats (Heideman 2000).

In males, high stress sensitivity is probably not as beneficial as in females. In reproductively active males experiencing constant stressful encounters with conspecifics that are also reproductively active (Morrison 1979; Morrison and Morrison 1981), a strong and chronic elevation of corticosterone levels might result if sensitivity to stress was as high as observed in females. Therefore, a delay in response may help avoid costs but guarantee the benefits of a corticosterone response in critical situations such as fights among males or chases by predators. The correlation between testis volume and circulating corticosterone levels implies both a direct link between reproductive activity and endocrine stress response and a capacity to modulate stress sensitivity with reproduction.

Seasonal differences in corticosterone

In order to maximize lifetime reproductive success, the reproductive cycle of many organisms is closely linked to resource availability and is therefore highly seasonal. In our study population, birth peaks of *A. jamaicensis* have been shown to precede periods of maximum fruiting activity of canopy trees on BCI (Kalko 1998), most likely to meet increased energetic demands of reproduction. The dry season period in our study was characterized by high reproductive activity in both males and females, whereas in the wet season the majority of males and all females were reproductively inactive. This raises the question of whether the corticosterone stress response in *A. jamaicensis* on BCI is primarily modulated directly by environmental factors or indirectly by reproduction. If environmental factors were the major driving force determining sensitivity of the HPA axis and the intensity of the stress response, then reproductively inactive individuals should show similar responses as reproductively active individuals in both temporal dynamics and intensity of stress response as a consequence of experiencing the same pressures of changes in fruit availability and climatic conditions, most importantly rainfall. Our data do not support this prediction, as dry season corticosterone levels of reproductively inactive females resembled those of reproductively inactive females in the wet season, while reproductive individuals experienced a significantly stronger endocrine stress response. In males, the pattern was less clear as all but one individual caught in the dry season were reproductively active and, as discussed above, reproduction in males appears to depress corticosterone. Overall, our data point towards a central influence of reproduction on the stress response mediated by corticosterone and they refute the idea that the stress response in our tropical study species is primarily modulated by environmental conditions.

Conclusions

In this study, reproductive condition rather than seasonality of the environment was found to influence the response of the endocrine HPA axis to stress in the common fruit-eating bat. Reproductively active females likely represent the energetically most heavily challenged part of a population. They are presumably exposed to a high risk of predation during late pregnancy. This risk is aggravated because females have only few offspring during their lifetime, with a maximum of two per year. It is likely that fitness protection by risk avoidance facilitation can explain the more rapid and stronger but less sustained corticosterone release in reproductively active compared to inactive females. Males, in contrast, experience a high frequency of social conflict during reproduction. Their corticosterone response was depressed in the short term, most likely to prevent costs of chronically elevated levels. This study is the first to show gender-specific strategies of stress modulation in a free-living, neotropical bat population that copes with conflicting demands of reproduction. Our results suggest that the less pronounced seasonality in tropical environments, which allows for multiple reproductive events per year, indirectly modulates the corticosterone stress response, while this response is directly modulated by reproduction. This may be achieved at least in part by an alteration of the sensitivity of the HPA axis, in the regulation of which many other factors (central and peripheral) are involved. Future studies should therefore address aspects such as variations in GC receptor expression, alterations in feedback loop activity, concentrations of GC-binding globulins in the plasma and effects of gonadal steroids on circulating GC concentrations.

Acknowledgements We are indebted to Nina Becker, Inga Geipel, Wolfgang Goymann, Nina Kriegisch, Chris Rothenwöhrer, Gabriele Wiest and the John Wingfield lab for help in the field and with analyses and to the Smithsonian Tropical Research Institute, Panama, for logistic support. Anne Goldizen, Stefan Jarau, Egbert Leigh, Jasmine Loveland and two anonymous reviewers gave valuable comments on the manuscript. This research was supported by the Arthur von Gwinner Foundation within the Max Planck Society, Friedrich Ebert Foundation (FES), German Academic Exchange Service (DAAD), German Science Foundation (DFG) and National Science Foundation (NSF). Experimentation and transport of biological samples were done holding appropriate permits and complied with the 'Principles of animal care', NIH publication no. 86-23, as well as with the current laws of both Panama and the European Union.

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